SOME NEW TYPES OF CHROMOSOME DISTRIBUTION AND THEIR RELATION TO SEX.[1]

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INTRODUCTON.

Since the discovery of the "accessory chromosome" in *Pyrrhocoris,* by Henking, this chromosome or its equivalent has been described in many other Hemiptera (Paulmier, Montgomery, Wilson and others) and also in many other tracheates—Orthoptera (McClung, Sutton and others), Coleoptera (Stevens), Myriapoda (Blackman and Medes) and Odonata (Lefevre and McGill). Wallace and Berry have also described it in the Arachnida. Essentially, the "accessory chromosome" is a spermatogonial chromosome, which is without a synaptic mate, and which divides in but one maturation division. The end result is that one half of the spermatozoa receive one more chromosome than the other half. The spermatozoa are therefore dimorphic in respect to the number of chromosomes.

A second type of dimorphism of the spermatozoa was discovered by Stevens ('05) in *Tenebrio* and by Wilson ('05) in *Lygæus, Cœnus* and several other genera of Hemiptera, and was afterward found by Stevens ('08) in the Diptera. In this type, both classes have the same number of chromosomes, but differ in that one class contains a large idiochromosome and the other a small one. These observers showed that the class containing

[1] My material was taken in large part by Professor E. B. Wilson on his collecting trip through the South and West, and it is due to this material that I have been able to make this comparative study. I have also received much valuable material from Rev. A. H. Manee, Southern Pines, N. C., besides collections which I have made in New Jersey, Massachusetts and Indiana. This material Mr. E. P. Van Duzee, of Buffalo, has kindly identified for me. Throughout the entire work, I have been much indebted to Professor Wilson for helpful suggestions and criticisms. I wish to thank Dr. Alexander Petrunkevitch for the privilege of working in his private laboratory at Short Hills, New Jersey, for five weeks during the spring of 1908. The U. S. Bureau of Fisheries also extended to me the privilege of working in the laboratory at Woods Hole, Massachusetts, during the summer of 1908.

the small idiochromosome produces males and the other class females. Wilson likewise established the fact that in forms having an accessory chromosome, the spermatozoa that contain this chromosome are female-producing, the others male-producing. In respect to sex-production, the accessory chromosome is, therefore, identical with the large idiochromosome. The homologue of the small idiochromosome is lacking.

The relation of the accessory chromosome to sex-production has been confirmed by the work of Montgomery ('06) on the Hemiptera heteroptera, of Stevens ('05 and '06) in the Coleoptera, of Boring ('07) on the Hemiptera homoptera, of Lefevre and McGill ('08) on *Anasa* and *Anax* (one of the Odonata), and of Wassilieff ('07) on the cockroach. It has also received confirmation in the recent work of Morgan ('08) and von Baehr ('08) in the parthenogenetic reproduction of *Phylloxera* and *Aphis*.

Wilson ('09) has described a third type in *Syromastes*. Gross ('04) showed that the accessory chromosome in this species is bivalent, but described and figured 22 chromosomes in both male and female cells. Wilson confirmed this for the male, but inferred that the female cells should contain 24 chromosomes or two more than the male, and he has since confirmed the correctness of this inference by direct observation. Hence, contrary to the assumption of Gross, both classes of spermatozoa must be functional, the class containing the bivalent accessory chromosome producing females, the others, males.

A fourth type was described by myself ('08) in a preliminary note on *Gelastocoris* (*Galgulus*). In this type there are five chromosomes which divide as univalents in the first maturation division. In the second division they do not divide, but four of the five pass to one pole and the other one to the opposite pole. In this form the female has three more chromosomes than the male, and the spermatozoa which contain the three extra chromosomes must therefore be the female-producing class.

A study of the Reduviidæ has brought to light several additional types here to be described. In *Diplocodus*, belonging to this family, occurs the second type with one large and one small idiochromosome. As in *Lygæus* and other forms, these divide

as univalents in the first division, but in the second, they come together in the middle of the metaphase plate to form a dyad. The two chromosomes of this dyad group separate, one passing to one pole and the other to the opposite pole.

Another and the first new type described in the present paper is found in *Fitchia,* where there are three chromosomes which divide as univalents in the first division, but in the second division arrange themselves in the form of a triad group, two members of which pass to one pole and one to the other.

In *Prionidus* and *Sinea,* we find a second new type in which there are four corresponding chromosomes. These four divide only in the first division. In the second, they are arranged in the form of a tetrad group, three members of which pass to one pole and one to the opposite one.

A third new type is present in *Acholla multispinosa.* Here there are six univalent chromosomes in the first division. My evidence is not absolutely conclusive in this case, but as far as it goes, it indicates that these six divide in the first division, but in the second, five of them pass, without dividing, to one pole and one to the other.

In all these cases the male and female chromosome groups differ correspondingly. In *Diplocodus* the male and female cells have the same number of chromosomes, but differ in that the female cells have a large idiochromosome and the male cells a small one. In *Fitchia,* the female has one more chromosome than the male; in *Prionidus* and *Sinea* two; in *Gelastocoris* three; and in *Acholla multispinosa* four. These facts prove that in each case one class of spermatozoa is female-producing and the other class male-producing.

The discovery of these types adds nothing new in principle to the theory of sex production as put forward by Wilson ('06), but they are perfectly consistent with it. *Acholla multispinosa,* in which the male seems to have the larger quantity, offers evidence against the interpretation that sex is determined by a quantitative relation of the chromatin.

In the preliminary note ('08) on *Gelastocoris,* I used the term "differential chromosomes" to refer to the chromosomes of the pentad group. Throughout the present paper, the same term

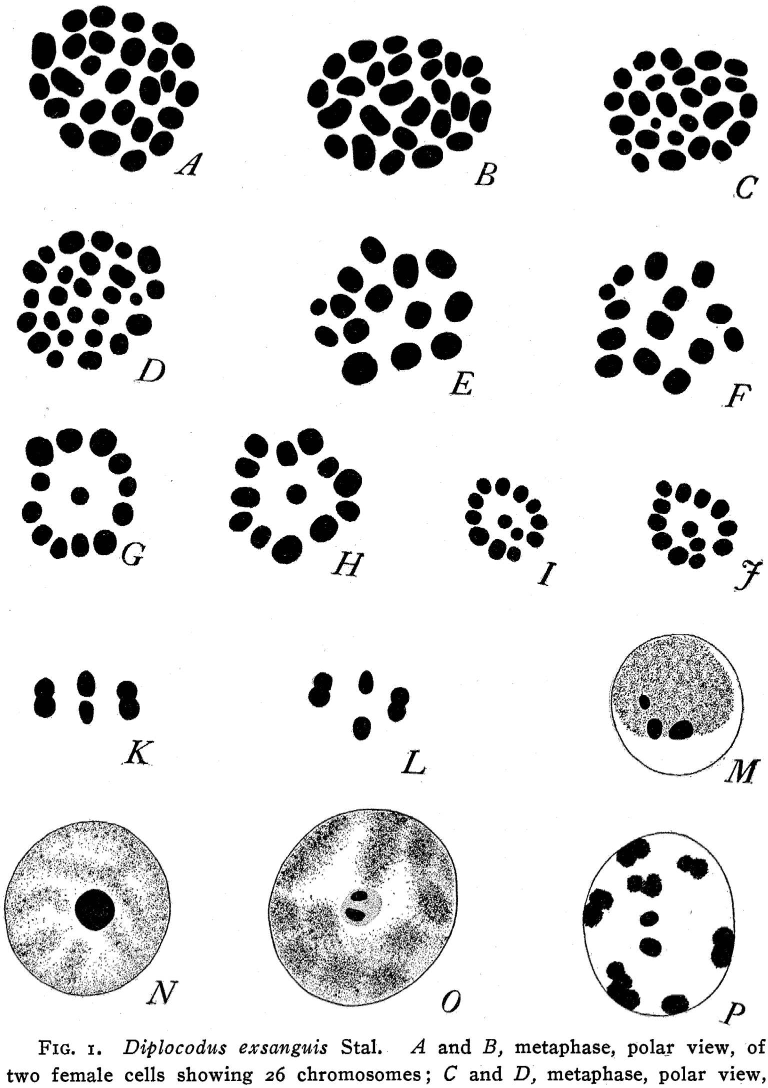


FIG. 1. *Diplocodus exsanguis* Stal. *A* and *B*, metaphase, polar view, of two female cells showing 26 chromosomes; *C* and *D*, metaphase, polar view, of two male cells, 26 chromosomes; *E* and *F*, two metaphase figures of the first maturation division, 14 chromosomes; *G* and *H*, two metaphase figures of the second maturation division, 13 chromosomes; *I* and *J*, two anaphases of the second division, taken from the same spindle; *K*, a side view of the second division, metaphase; *L*, side view of the second division, early anaphase, showing the early separation of the idiochromosomes; *M*, the early growth period, about contraction-phase. Two of the dark bodies are probably the idiochromosomes and the other the plasmosome; *N* and *O*, two drawings from approximately the same stage in the growth period. In *N* the plas-

will be used in reference to the chromosomes of the triad, tetrad, pentad and hexad groups.

DESCRIPTIVE.

Diplocodus exsanguis Stal.

In *Diplocodus* we find a typical pair of idiochromosomes as described by Wilson ('05) for several species of Hemiptera. A description is included here mainly to show the range of variability in chromosome distribution found within the limits of a single family of Hemiptera. There are 26 chromosomes in the somatic groups of both male and female cells (Fig. 1, *A, B, C* and *D*). While there is very little difference in size in the idiochromosomes, there is one chromosome in the male group, noticeably smaller than the others, which without doubt, is the small idiochromosome. As the idiochromosomes are separate in the first division, the metaphase plate of the first division shows 14 chromosomes (Fig. 1, *E* and *F*), one more than half the spermatogonial number. In these figures, the small idiochromosome can be readily identified, but one cannot always be sure of the large one. Both divide in this division. In the second division, there is a rearrangement of the chromosomes, 12 taking up the position of a ring with the idiochromosomes, one above the other, in the center (Fig. 1, *I* and *J*, side views of the second division, metaphase). A polar view of the metaphase accordingly shows only 13 chromosomes. Wilson figures them as coming into close apposition at this time to form an asymmetrical dyad. While I have not examined a large number of cases in *Diplocodus,* they, here, seem not to fuse, but only to come very close together as shown in Fig. 1, *I*. Fig. 1, *J*, shows that their separation precedes the division of the chromosomes in the ring, as is also the case in the forms described by Wilson. Fig. 1, *K* and *L,* represent two anaphases taken from the same spindle.

The history of the idiochromosomes during the growth period has not been fully traced. In the contraction-phase (Fig. 1, *M*),

mosome stains intensely black, while in *O* it is pale and the idiochromosomes can be seen lying embedded in it; *P* is a prophase figure of the first division, showing the idiochromosomes in marked contrast to the looser masses of chromatin condensing to form the remaining chromosomes. The enlargement is 3,726 diameters.

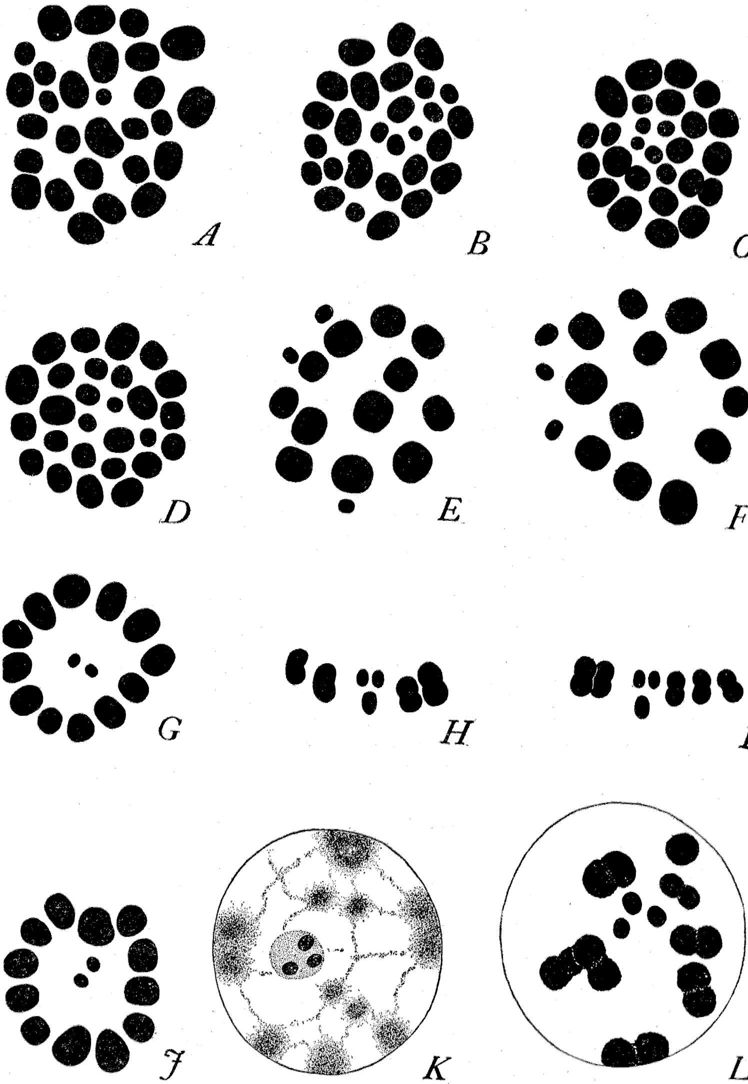


FIG. 2. *Fitchia spinosula* Stal. *A* and *B*, two female cells, metaphase, polar view, 28 chromosomes; *C* and *D*, two male cells, metaphase, 27 chromosomes; *E* and *F*, first maturation division, metaphase, showing 15 chromosomes —the three smallest ones are the differential chromosomes; *G* and *J*, polar views of the second division, metaphase—only two of the differential chromosomes are shown, as the third lies beneath these two; *H* and *I*, side views of the second division, metaphase—the triad group, formed by the three differential chromosomes, in the center; *K* gives the typical appearance of the plasmosome and differential chromosomes during the greater part of the growth

three dark bodies are sometimes present. Judging from the conditions found in Fig. 1, *N* and *O*, and from the relation of the chromosomes and plasmosome in *Prionidus*, where it has been fully worked out, it seems very probable that two of these dark bodies may be the idiochromosomes and the other, the plasmosome. Fig. 1, *N* and *O*, represent about the same stage in the growth period, but are entirely different in appearance. In *N*, the whole nucleolus stains intensely black, but again as shown in *O*, we see a pale plasmosome with the idiochromosomes embedded in it. There is no doubt but that the nucleolus in both cases represents the same thing. The only difference is in the staining capacity of the plasmosome. What causes this difference is impossible to say. However, it is very probable that the plasmosome is an active agent and as such is constantly undergoing changes, either chemical or physical, or both. In the prophase of the first division (Fig. 1, *P*) the plasmosome breaks down and the idiochromosomes stand out plainly while the other chromosomes are condensing. In the cases described by Wilson ('05) the idiochromosomes were attached to a plasmosome and not actually embedded in it as we find in *Diplocodus*.

Fitchia spinosula Stal.

In forms where the accessory chromosome is a single unpaired element, this chromosome remains throughout the growth period as a chromosome nucleolus, and since it divides in but one maturation mitosis, half the spermatozoa receive one more chromosome than the other. As already stated, these spermatozoa produce females, the others males, since the female somatic cells contain one more chromosome than the male.

In *Fitchia*, an exactly similar result is produced, yet, the manner in which the unequal distribution is brought about is entirely different. The female cells (Fig. 2, *A* and *B*) have 28 chromosomes, and the male cells (Fig. 2, *C* and *D*), 27. In neither of these groups can the differential chromosomes be recognized with certainty. The first division (Fig. 2, *E* and *F*) shows 15

period; *L*, prophase of the first maturation division—the plasmosome has disappeared and the chromosomes are forming. The enlargement is 3,726 diameters.

chromosomes, three of which are much smaller than the others, and prove to be the univalent differential chromosomes. Here they lie on the periphery. All the chromosomes divide equally as the same total number (15) is found in the metaphase plate of the second division. Now, however, the arrangement is different, the 12 larger chromosomes forming an irregular ring within which lie the three differential chromosomes in the form of a triad group, similar in arrangement to the pentad group in *Gelastocoris* (*Galgulus*). Two of these three, nearly or quite of the same size, lie in the same plane, while the third, which is a little larger, lies either above or below them on the other side of the equatorial plane (Fig. 2, *I* and *J*, two side views of the second division, metaphase). While my material does not show the anaphases of this division, the relations in the male and female cells and a comparison between this and the other two species, which show the same type of distribution and where anaphases are present, leave little doubt as to the manner of division. The 12 chromosomes in the ring divide equally, while the members of the triad group do not divide individually, but the group as a whole separates so that the two chromosomes pass to one pole and the one to the other. Two classes of spermatozoa containing respectively 13 and 14 chromosomes are thus produced. Since the female number is 28 and the male 27, it is evident that the reduced number in the egg must be 14, and that females are produced upon fertilization of the egg by the 14-chromosome class; males upon fertilization by the 13-chromosome class, as follows:

Egg 14 plus spermatozoön 13 = 27 (♂),
Egg 14 plus spermatozoön 14 = 28 (♀).

Although the end result is the same as in those species with an odd chromosome, this gives us a new type of chromosome distribution, which is the first step in a series, which bridges the gap between the relations found in *Diplocodus*, on the one hand, and *Gelastocoris* (*Galgulus*) on the other.

The history of the differential chromosomes in the growth period has not been followed in detail, but during the greater part of this time, a pale plasmosome is present and embedded

in it are the three differential chromosomes as densely staining compact bodies (Fig. 2, *K*). In the prophase of the first division, the plasmosome disappears and the differential chromosomes remain (Fig. 2, *L*).

Rocconota annulicornis Stal.

With the exception of the size relations of the differential chromosomes, we find practically the same conditions in this form as in *Fitchia.* The spermatogonial cells (Fig. 3, *A* and *B*) show 27 chromosomes. Unfortunately no good female groups were present in my material, but since the relations are so similar to those found in *Fitchia,* there seems little doubt but that it contains one more chromosome than the male. In the first division, metaphase plate (Fig. 3, *C* and *D*), are 15 chromosomes, three of which are distinctly smaller than the remaining twelve, and which prove to be the differential chromosomes. All divide equally in this division. The characteristic regrouping in the second division again occurs. The 12 larger chromosomes form an irregular ring with the triad group, made up of the three differential chromosomes in the center (Fig. 3, *G* and *H*, side views of the second division, metaphase). Here all three differential chromosomes are practically of the same size. The chromosomes in the ring divide equally, while those of the triad group separate as in *Fitchia,* so that two pass to one pole and one to the other. The two classes of spermatozoa thus produced, are essentially similar to those of *Fitchia,* and are respectively male and female producing. Fig. 3, *I* and *J,* are two anaphases taken from the same spindle which show the unequal distribution of the chromosomes, 14 passing to one pole and 13 to the other.

Throughout the growth period, from shortly after synapsis to the prophase of the first division, there is present a distinct pale plasmosome, in which are embedded the three differential chromosomes (Fig. 3, *K* and *L*). The exact stage in which the plasmosome and chromosomes make their appearance could not be determined. Fig. 3, *M,* represents the prophase of the first division, where the plasmosome has disappeared. The differential chromosomes stand out in marked contrast to the looser

masses of chromotin condensing to form the remaining chromosomes.

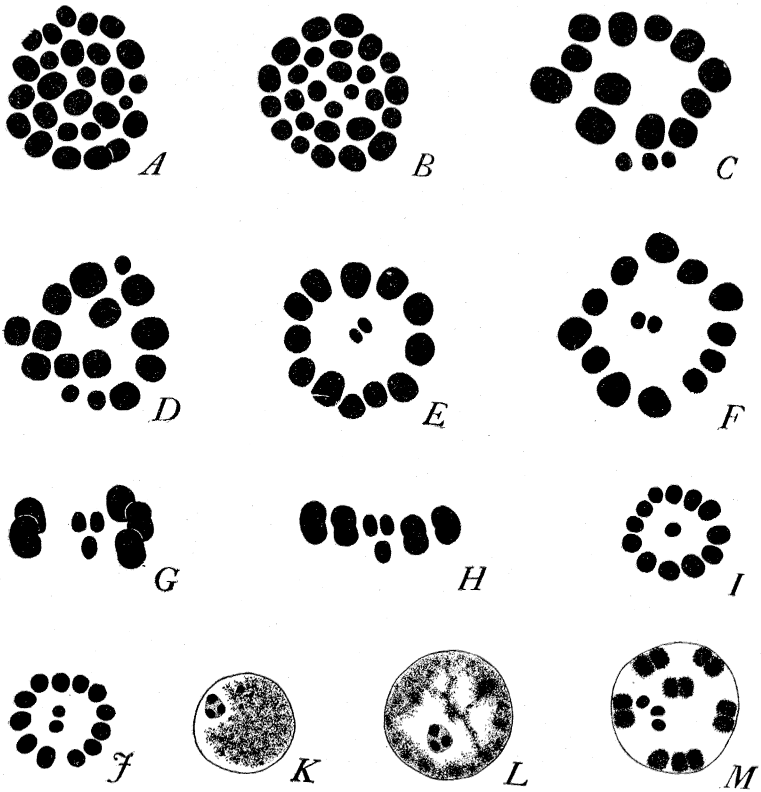


FIG. 3. *Rocconota annulicornis* Stal. *A* and *B*, male chromosome-groups, 27 chromosomes; *C* and *D*, polar views of the first spermatocyte division, showing 15 chromosomes—the three small ones are the differential chromosomes; *E* and *F*, polar views of the second division—only two of the three differential chromosomes are shown, as the third lies below the two; *G* and *H*, side views of the second division metaphase, showing arrangement of the triad group of differential chromosomes; *I* and *J*, two anaphases of the second division taken from the same spindle, which show the manner of separation of the triad group; *K*, a stage in the early growth period, showing the pale plasmosome and the three differential chromosomes embedded in it; *L*, a stage giving the typical appearance of the nucleus during the greater part of the growth period; *M*, prophase of the first division—the plasmosome has disappeared and the three differential chromosomes are easily recognizable. *K*, *L* and *M* are magnified 2,411 diameters; the remainder 3,726 diameters.

Conorhinus sanguisugus Lec.[1]

Conorhinus gives an additional verification of the type described in *Fitchia.* The number and size relations of the chromosomes are somewhat different. The spermatogonial divisions (Fig. 4, *A* and *B*) show 23 chromosomes, two of which are distinctly smaller than the others. In the metaphase plate of the first division (Fig. 4, *C* and *D*) there are 13 chromosomes, ten of which must be bivalent and three univalent as there are 23 in the spermotogonia. In this division, contrary to what is found in all other cases, the bivalent chromosomes form an irregular ring and the differential chromosomes, which are easily recognized by their size relations, take up a central position, but all lie in one plane. All the chromosomes divide equally in this division, so that each secondary spermatocyte receives 13 chromosomes, the arrangement of which is somewhat different from that in the first division. There are ten in the ring, but the differential chromosomes which occupy a central position, show a regrouping as in *Fitchia.* The large and one small one lie in the same plane, but the second small one is either above or below the other two, forming the characteristic triad group (Fig. 4, *G* and *H,* side views of the second division, metaphase). The ten chromosomes in the ring divide equally, while the chromosomes of the triad group separate, two going to one pole and the other to the opposite pole. Fig. 4, *I* and *J*, representing two anaphases from the same spindle, show the chromatin content of the two class of spermatozoa, which contain respectively 13 and 14 chromosomes.

The size relations of the differential chromosomes in the three forms illustrating this type of distribution are different in each case. In *Fitchia* the one which goes to the male-producing pole is slightly larger than the other two. In *Rocconota* all three are practically of the same size and in *Conorhinus,* one of the two which goes to the female-producing pole is larger than the other two.

The history of the differential chromosomes in the growth period has been followed as far as material permits. Sometimes

[1] This identification is somewhat doubtful as all my specimens are immature.

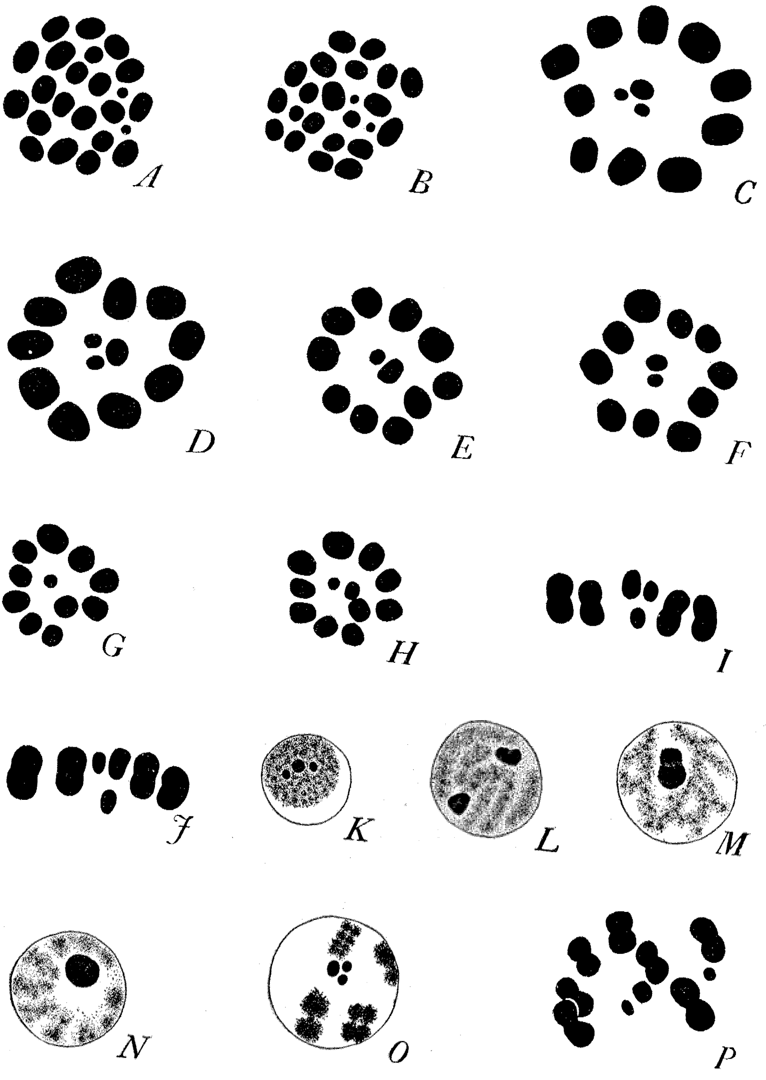


FIG. 4. *Conorhinus sanguisugus* Lec. *A* and *B*, male chromosome-groups, 23 chromosomes; *C* and *D*, polar views of the first maturation division, metaphase—the three differential chromosomes lie in the middle of the irregular ring; *E* and *F*, polar views of the second mitosis—only two differential chromosomes are shown; *G* and *H*, two anaphases of the second division taken from the same spindle and which show the manner in which the triad group separates; *I* and *J*, side views of the second division, metaphase, showing arrangement of the triad group; *K*, a stage in the early growth period, about contraction-phase—the three dark bodies are the three differential chromo-

they appear as separate and distinct individuals in the contraction phase (Fig. 4, *K*). A little later (Fig. 4, *L*) when the chromatin is scattered about through the nucleus, two dark bodies, nearly equal in size, are present. It is very probable that they are both plasmosomes. In one of them, three denser bodies can be faintly, yet unmistakably seen. These are no doubt the three differential chromosomes. The two plasmosomes seem to fuse into one body as in Fig. 4, *M*, and later condense into a somewhat spherical mass (Fig. 4, *N*), which persists throughout the greater part of the growth period. The plasmosome goes to pieces in the prophase of the first division and the differential chromosomes appear at the same time. While in Fig. 4, *M* and *N*, the differential chromosomes cannot be seen, there is little doubt but that they are present in the plasmosome. From these figures alone, it would be impossible to make the above statement, but in view of the condition in *Prionidus*, which is described later, I believe the correct interpretation has been given.

Prionidus cristatus Linn.

In his suggestive papers of '01 and '06, Montgomery described in *Prionidus*, the rest stage of the spermatogonia, the spermatogonial divisions and the rest stage of the primary spermatocytes. For the main part, my observations support his, but as we shall see, he was led to some erroneous conclusions because he did not follow the chromosomes through the first and second divisions. He figures 26 chromosomes in the spermatogonial divisions, and in describing these ('06) he says, "Of the 26 chromosomes two are much larger and two much smaller than the others. All these are found on careful inspection to be arrangeable into a series of pairs, in which the two components

somes; *L*, a stage after synapsis, showing two plasmosomes, in one of which the three differential chromosomes can be faintly seen; *M*, a stage a little later than *L*, showing fusion of the two plasmosomes; *N* shows the complete fusion of the plasmosomes and the typical appearance of the nucleus throughout the growth period; *O*, prophase of the first division—the plasmosome has disappeared—the three densely staining bodies are the differential chromosomes; *P*, a later prophase, just before the chromosomes arrange themselves in the equatorial plate. *K, L, M, N* and *O* are magnified 2,411 diameters; the remainder 3,726 diameters.

of each pair are of approximately equal volume except the two marked *K, k*. There is probably no monosome because the number is an even one."

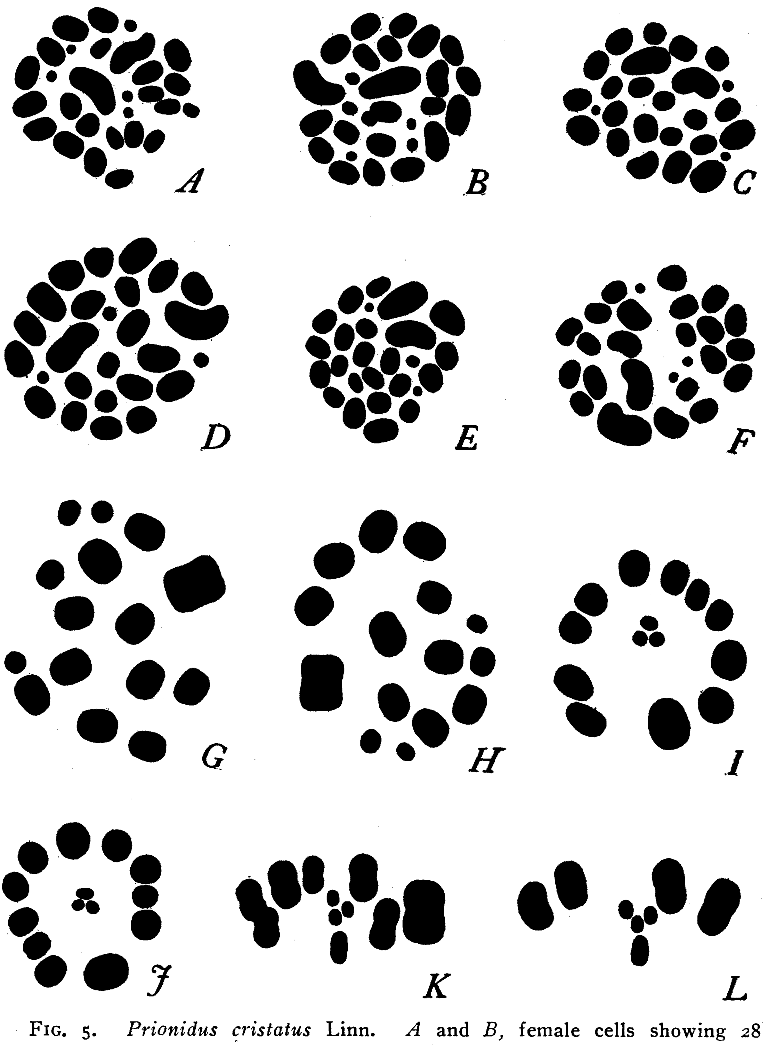


FIG. 5. *Prionidus cristatus* Linn. *A* and *B*, female cells showing 28 chromosomes—the six smallest ones are the differential chromosomes; *C, D, E* and *F*, male cells, 26 chromosomes—only three of the four differential chromosomes can be recognized with certainty—they are the three smallest ones; *G* and *H*, polar views of the first maturation division, 15 chromosomes—the four smallest ones are the differential chromosomes; *I* and *J*, polar views of the second division, metaphase—only three differential chromosomes are shown; *K* and *L*, side views of the second division, metaphase, showing the arrangement of the tetrad group. The enlargement is 3,726 diameters.

My figures of the male groups (Fig. 5, *C, D, E* and *F*) agree both in number and in size relations with those of Montgomery. There are 26 chromosomes, two of which are larger and three smaller than the rest. Although Montgomery's figures in both papers ('01 and '06) show three small chromosomes, he describes three small ones in the first paper and only two in the latter. The number of chromosomes and their behavior in the first and second divisions give an explanation of the size relations in the spermatogonia.

The female groups (Fig. 5, *A* and *B*) show 28 chromosomes, six of which are distinctly smaller than the others.

In the first maturation division metaphase, 15 chromosomes are present, four of which can be easily recognized as smaller than the remaining ones and one of the four is decidedly larger than the other three. There is no definite arrangement in these figures. All the chromosomes divide equally, so that accordingly each secondary spermatocyte receives 15 chromosomes, the arrangement of which is entirely different. The eleven larger chromosomes form an irregular ring while the four smaller ones, which prove to be the differential chromosomes, form a tetrad-group in the center (Fig. 5, *K* and *L,* two side views of the second division, metaphase). Unfortunately no anaphases of the second division were present, but the number and size relations in the male and female groups and the analogy between the behavior here and in *Sinea,* which is described later, leave no doubt as to the manner in which the tetrad-group separates. The eleven chromosomes in the ring divide equally, while the members of the tetrad-group do not divide, but the group as a whole separates so that the three smaller ones pass to one pole and the larger one passes to the other pole. Two classes of spermatozoa, containing 12 and 14 chromosomes, respectively, are thus produced. Accordingly the 14-chromosome class contains three small chromosomes while the 12-chromosome class would have none, as the differential chromosome which goes to this pole is as large as one half of one of the smaller dyads (see Fig. 5, *K* and *L*). If the 14-chromosome class meets the egg with 14 chromosomes, three of which are small, we would expect 28 chromosomes in the offspring, six of which should be small.

This number and the size relations are exactly what is found in the female cells (Fig. 5, *A* and *B*). Again, if the 12-chromosome class meets the same egg we would expect 26 chromosomes in the offspring, three of which should be small, and this number and relation is confirmed by the figures of the male cells (Fig. 5, *C, D, E* and *F*). From the facts at hand, it is evident that the reduced number of chromosomes in the egg is 14 and that three of these are decidedly smaller than the rest. Females must therefore be produced upon fertilization of the egg by the 14-chromosome class of spermatozoa; males by the 12-chromosome class.

Egg 14 plus spermatozoön 12 = 26 (♂),
Egg 14 plus spermatozoön 14 = 28 (♀).

From the above observations, it becomes apparent that the three small chromosomes and one other, which is not recognizable in the spermatogonial divisions, do not pair at synapsis. This gives an explanation of Montgomery's unequal pair.

Prionidus thus gives us a second new type of chromosome distribution and presents the intermediate stage between the types illustrated by *Fitchia* and *Gelastocoris.*

Montgomery has described only the rest stage of the growth period. In his earlier paper ('01) he says: "In the rest stage of the spermatocyte are found four chromatin nucleoli attached to the true nucleolus. One of these is longer than the others and rod-shaped." Later ('06) he describes this stage somewhat differently. "In the complete rest stage of the spermatocytes are found three or four safrininophilous bodies attached to the surface of a large more or less central plasmosome. They are of unequal volumes; and when there are three of them, each appears bipartite, while when there are four, the smallest are each unipartite. Perhaps as in *Sinea,* these relations are to be interpreted as three bivalent diplosomes, the smallest of which may sometimes have its parts separated."

I have followed the history of the differential chromosomes during the growth period somewhat in detail as the material proved favorable for this purpose. Between the last spermatogonial division and synapsis, a pale plasmosome persists. At

the contraction-phase (Plate 1, *A*), there is present in addition to the plasmosome, a deeply staining body, which proves to be the first appearance of the differential chromosomes. In the spireme stage (Plate 1, *B*), shortly after synapsis, the two bodies are present, but the chromatic mass is more spherical. A little later (Plate 1, *C*), the chromatic body has become broken up and a plasmosome has formed around it. The next stage (Plate 1, *D*) shows the two bodies in contact and beginning to fuse. About this time the chromatic mass breaks up for the first time into its constituent elements, the four differential chromosomes, the larger of which is noticeably elongate (Plate 1, *E*). The two plasmosomes fuse completely to form a large more or less spherical one (Plate 1, *F*). Plate 1, *G*, shows the typical appearance of the nucleus throughout the greater part of the growth period. The larger differential chromosome shortens into a somewhat spherical form. All of them lie embedded in the pale plasmosome and not in contact with it as described by Montgomery. Nor do any of them, at any time, show an appearance of being bipartite and their later behavior in the maturation divisions shows conclusively that all four are univalent. As we shall see later, Montgomery's idea as to the relations in *Sinea* were incorrect, and this accounts in part for the wrong interpretation in *Prionidus*. In the prophase of the first division, when the chromatin is beginning to condense to form the chromosomes, the plasmosome disappears and the differential chromosomes remain.

In *Syromastes,* Wilson ('09) describes the same numerical difference in the male and female somatic cells as here described for *Prionidus*. The difference in that case, however, is brought about by two chromosomes which act as a unit and which divide in only one division.

Sinea diadema Fabr.

Montgomery ('01 and '06) described in part the behavior of the chromosomes in a species which he called "*Sinea diadema,*" but the relations which I find in this species are entirely different from those which he described. There is, therefore, but little doubt that he studied a different species. This again em-

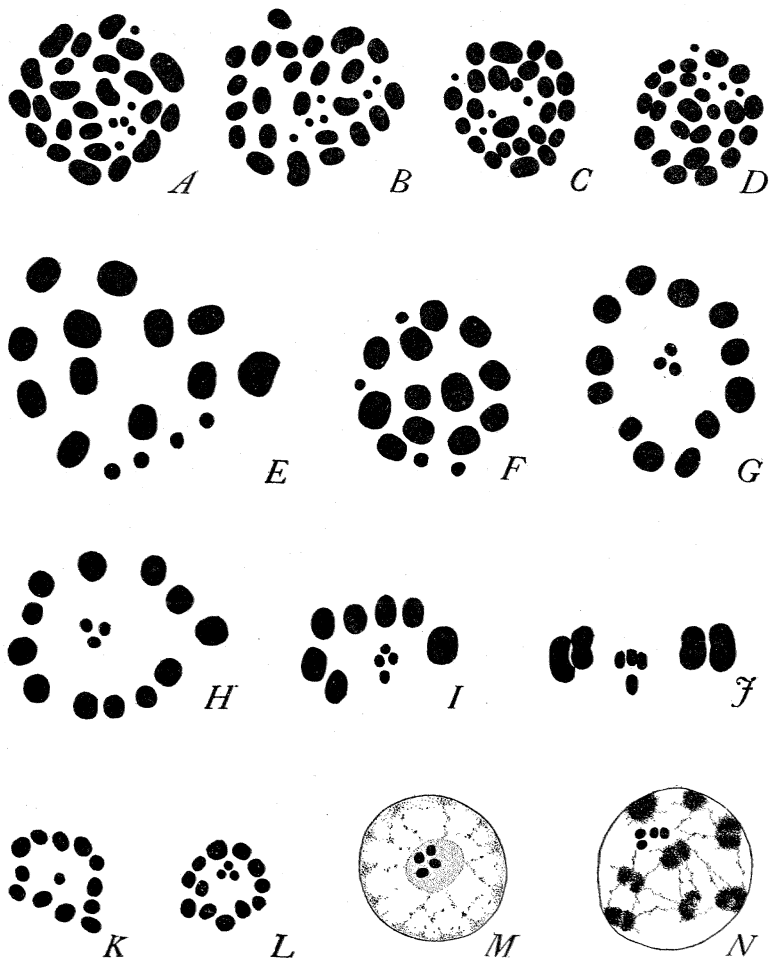


FIG. 6. *Sinea diadema* Fabr. *A* and *B*, female groups, metaphase figures, showing 30 chromosomes—the six small ones are the differential chromosomes; *C* and *D*, male groups, showing 28 chromosomes—the four small ones are the differential chromosomes; *E* and *F*, polar views of the first maturation division, metaphase, 16 chromosomes—the four small ones are the univalent differential chromosomes; *G* and *H*, polar views of the second division, metaphase—only three differential chromosomes are shown, as the fourth lies below the three; *I* and *J*, side views of the second division, metaphase, showing arrangement of the tetrad group; *K* and *L*, two anaphases of the second division, showing the manner in which the tetrad group separates, and the number of chromosomes in each of the two classes of spermatozoa; *M* gives the typical appearance of the plasmosome and differential chromosomes during the greater part of the growth period; *N*, a prophase of the first division after the plasmosome has disappeared—the four small condensed bodies are the differential chromosomes. *A–L* are magnified 3,726 diameters; *M* and *N* 2,411 diameters.

phasizes the necessity of keeping all specimens for future identification. It seems very probable that the species which Montgomery called "*Sinea diadema*" was *Acholla multispinosa*, for the first maturation division in *Acholla multispinosa* agrees exactly with that which Montgomery figured for *Sinea*.

The behavior of the chromosomes in *Sinea* is practically the same as in *Prionidus* and gives a beautiful confirmation of the results there described. In the female cell (Fig. 6, *A* and *B*), 30 chromosomes are distinctly visible, six of which are much smaller than the others. The male cells (Fig. 6, *C* and *D*) show 28 chromosomes, two less than the female number and four of these are smaller than the rest. There are 16 chromosomes in the metaphase of the first maturation division (Fig. 6, *E* and *F*), and since there are 28 chromosomes in the spermatogonia, 12 of these must be bivalent and four univalent. All the chromosomes divide equally in the first division. Hence the equatorial plate of the second division shows 16 chromosomes, but as usual, their arrangement is different. In the first division, the small chromosomes are peripheral, while in the second, they are central and are arranged in a tetrad-group, as are the four differential chromosomes in *Prionidus*. Fig. 6, *I* and *J*, show two side views of the second division, metaphase, with the tetrad group in the middle. In *Prionidus*, one chromosome of the tetrad group is larger than the other three, while in *Sinea* all four are practically the same size. In the second division, the chromosomes in the ring divide, while the tetrad group separates so that the three chromosomes above go to one pole and the one below to the other. Fig. 6, *K* and *L*, are two anaphases, showing the manner of separation. Two classes of spermatozoa are thus formed, containing 13 and 15 chromosomes, respectively. Judging from these facts, the reduced female group contains 15 chromosomes and females are produced upon fertilization by the 15-chromosome class of spermatozoa; males by the 13-chromosome class.

Egg 15 plus spermatozoön 13 = 28 (♂),
Egg 15 plus spermatozoön 15 = 30 (♀).

The size relations of the differential chromosomes which make

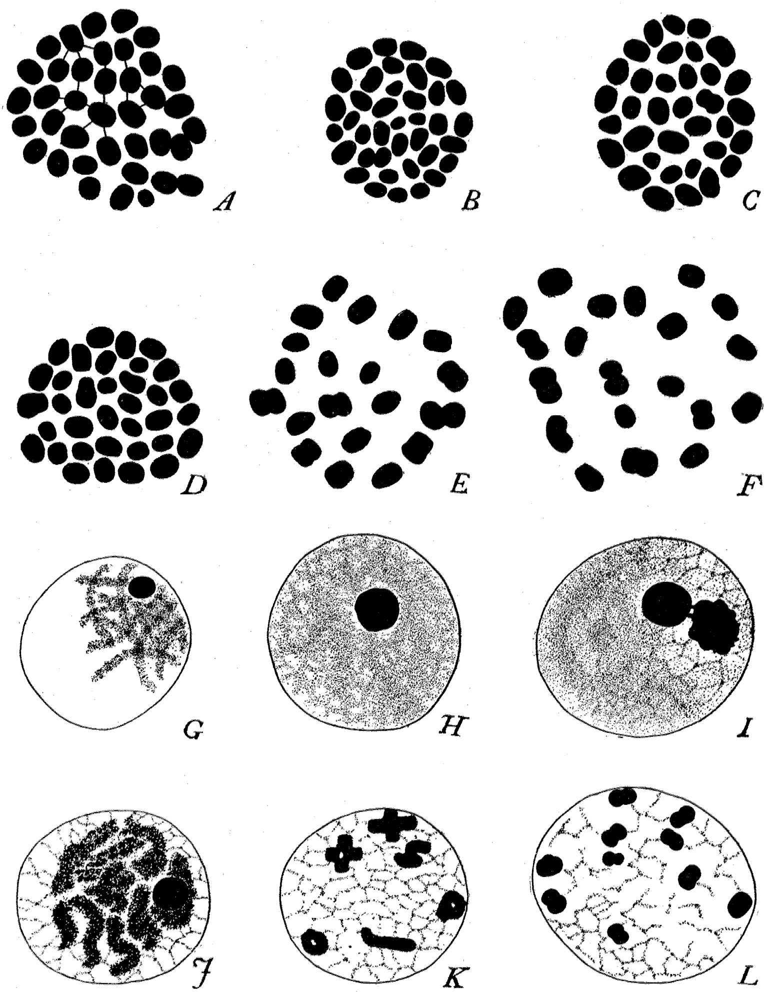


FIG. 7. *Gelastocoris* (*Galgulus*) *oculatus Fabr.* *A* and *B*, female groups, metaphase plates, showing 38 chromosomes; *C* and *D*, male groups, showing 35 chromosomes; *E* and *F*, polar views of the first maturation division, metaphase—the differential chromosomes are indistinguishable; *G*, a stage shortly after synapsis, showing the presence of the nucleolus; *H*, typical appearance of the nucleus and nucleolus throughout the growth period; *I*, a very early prophase of the first division—the contents of the nucleolus have been thrown out into the nucleus; *J*, prophase of the first division, when the chromosomes are just beginning to form—the plasmosome is still present, although apparently smaller; *K*, prophase of the first division—the chromosomes are condensing and the plasmosome has disappeared; *L*, late prophase of the first division—the chromosomes are formed, but have not taken up their position in the equatorial plate. *A*, *B*, *C* and *D* are magnified 3,105 diameters; *E* and *F* 2,009 diameters and *G*, *H*, *I*, *J*, *K* and *L* 1,297 diameters.

them easily recognizable in the male and female cells, help to confirm the above statement. Six are present in the female and only four in the male, and this is what we would expect, since one class of spermatozoa has three small chromosomes and other one.

The differential chromosomes have not been followed throughout the entire growth period. Fig. 6, *M*, shows the appearance of the nucleus during the greater part of this period. A large pale plasmosome is present in which are embedded the four differential chromosomes. The condition is almost exactly analogous to that found in *Prionidus*. In the prophase of the first division, the plasmosome disappears and the four differential chromosomes stand out clearly as condensed deeply staining bodies while the other chromosomes are condensing.

Gelastocoris (*Galgulus*) *oculatus* Fabr.

In a preliminary note ('08), I described a new type of sexual difference in the chromosome groups of *Gelastocoris oculatus*. At that time my material was limited and some of my conclusions were supported by only a few observations. Since then I have obtained new material and am now in a position to reaffirm all that was stated in the former paper. My figures of the first division showed among the chromosomes one or two minute dark bodies resembling yolk granules. As stated at that time, a doubt might be raised as to whether these bodies were chromosomes. However, my opinion as stated, was supported by the facts that the number of chromosomes in the prophase of the first division before the nuclear wall broke down, was always 20, and that the metaphase plate of the second division invariably showed 20 chromosomes. In my new material, I have found several metaphase plates which show 20 chromosomes without any granules whatever (Fig. 7, *K* and *L*). As the spermatogonial number is 35, 15 of the 20 must be bivalent and five univalent. There is no definite arrangement of the chromosomes in this division, but sometimes 15 are in a more or less irregular ring with the other five in the center, as is shown in Fig. 7, *L*. Whether these five are the same ones which form the pentad group in the second

division is impossible to say. All the chromosomes divide equally so that each secondary spermatocyte receives 20 chromosomes. No reconstruction period follows. (Fig. 8 and 9 are the same as in the preliminary paper, but Fig. 7 is new with the exception of *A*.)

My former description of the second division seems adequate, and as I have nothing new to add, I will quote it as there described.

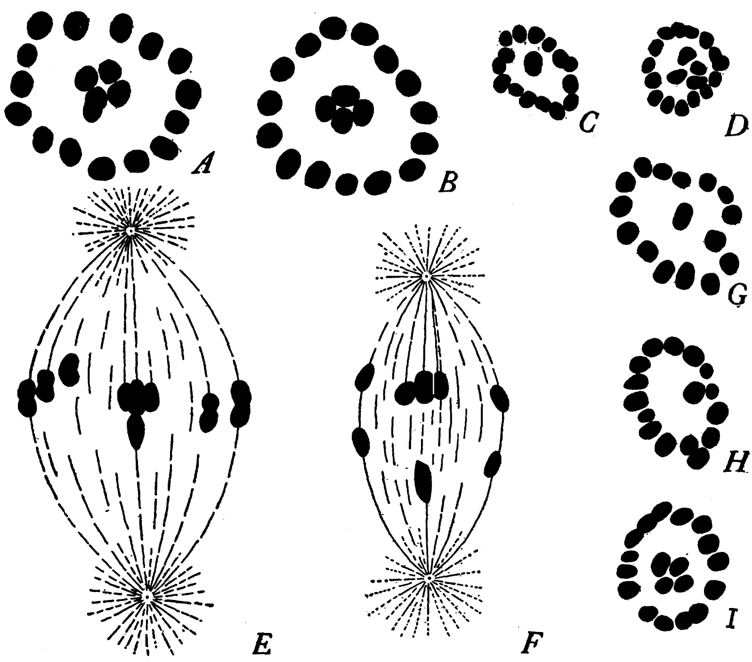


FIG. 8. *Galastocoris* (*Galgulus*) *oculatus* Fabr. *A* and *B*, metaphase figures of the second spermatocyte division, polar view, showing the ring of 15 chromosomes and the pentad group in the center—in *B*, the chromosome beneath the four group could not be shown without displacing it; *C* and *D*, late anaphases of the second division, polar view, showing the unequal distribution of the chromosomes; *E*, side view of the second division, metaphase, showing the typical arrangement and position of the pentad group; *F*, side view of the early anaphase, second division, showing the manner in which the chromosomes of the pentad group separate—the spindle in both *E* and *F* is diagrammatic; *G*, *H* and *I*, early anaphases of the second division. All drawings are made on the same scale and magnified 3,105 diameters.

"The second division which follows immediately after the first, shows a remarkable regrouping of certain of the chromosomes.

Fifteen of the 20 take up the position of a ring, within which is a definite compound element formed by the remaining five. These are now arranged in a pentad group, which always shows the same composition and occupies the same position. Four of these five chromosomes are grouped very closely together and lie in one plane, while the other one is either above or below this group of four, lying close to them on the other side of the equatorial plane (Fig. 8, *A*, polar view of the equatorial plate; Fig. 8, *E*, and Fig. 9, *C*, side views). The 15 chromosomes in the ring divide equally, while the chromosomes of the central pentad do not divide individually, but the group as a whole separates in such a manner that one chromosome passes to one pole and the other four to the other pole (Fig. 8, *F*, and Fig. 9, *D* and *F*). Two classes of spermatozoa are thus formed, which contain 16 and 19 chromosomes respectively. The early anaphase illustrating these two classes is shown in Fig. 8, *G*, *H* and *I*, and Fig. 9, *B*; the later anaphase in Fig. 8, *C* and *D*."

In my previous paper, I also stated somewhat cautiously that 35 was the number of chromosomes in the spermatogonia, as the count was made in only two cells. Fig. 7, *C* and *D*, shows two new groups and I have counted the chromosomes in several other cells, each case showing the same number. At present, I have, therefore, no hesitation is saying that 35 is the number of chromosomes found in the male cells. The female number (oögonial or follicle cells) is 38 (Fig. 7, *A* and *B*). From these facts it is evident that the reduced female group must contain 19 chromosomes; and that accordingly females are produced upon fertilization by the 19-chromosome class of spermatozoa; males upon fertilization by the 16-chromosome class.

Egg 19 plus spermatozoön 16 = 35 (♂),
Egg 19 plus spermatozoön 19 = 38 (♀).

During the growth period, between synapsis and the formation of the chromosomes preparatory to the first spermatocyte division, a large deeply staining body persists, which is more or less comparable to the nucleolus of the forms previously described. In the preliminary note, I suggested the possibility that this body

contained the five differential chromosomes. Further study has thrown very little light upon the question. Fig. 7, *E. F. G* and *H,* represents its history as far as I have traced it. *E* shows it shortly after synapsis; *F* gives its typical apparance when the cell has reached its maximum size; *G* represents a stage toward the

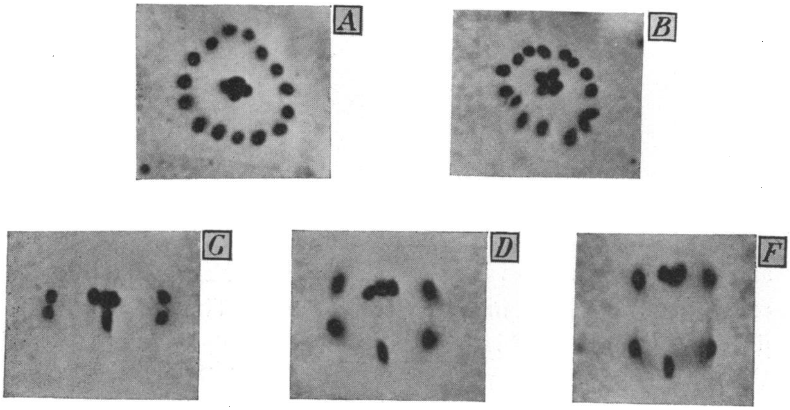


FIG. 9. *Gelastocoris* (*Galgulus*) *oculatus* Fabr. *A,* metaphase figure of the second division, polar view, showing the ring of 15 and the four chromosomes of the pentad group near the middle—the fifth chromosome of this group could not be shown, as it lies beneath the four; *B,* early anaphase of the second division, polar view, showing the 19 chromosomes which go to one pole; *C,* metaphase of the second division, side view, showing the typical position and arrangement of the chromosomes of the pentad group; *D* and *F,* anaphases of the second division, side view, showing the manner of separation of the pentad group, four chromosomes of which go to the one pole and one to the other—only three chromosomes of the four group show, as all of them do not lie in the same plane. The photographic enlargement is 1,500 diameters.

end of the growth period and before the chromosomes begin to form. At this time the contents are thrown out into the nucleus and it is probable that the part thrown out is chromatin as it appears to take part in the formation of the chromosome a little later. After the contents are thrown out, the nucleolus does not entirely disappear until the chromosomes are well formed (Fig. 7, *I*). As the five differential chromosomes cannot be identified before the second maturation division, it is impossible to trace their earlier history with accuracy. It is very evident, however, that if they are in the nucleolus they do not come out of it as condensed and separate individuals as they do in the other cases

described. No direct evidence is at hand, yet I still think there is a possibility that the deeply staining body is a plasmosome in which are the five differential chromosomes. While reasoning by analogy may not always be a safe method, the above interpretation, it seems to me, is supported by a comparison of the nucleolus here with those in Fig. 1, *N* (*Diplocodus*), and Fig. 4, *N* (*Conorhinus*). In these two figures the nucleolus stains as intensely black as in *Gelastocoris,* yet it is evident that the differential chromosomes are present, although invisible.

Acholla multispinosa De Geer.

Montgomery has also described in part the spermatogenesis of a form which he called "*Acholla multispinosa,*" but according to Van Duzee's identification he was here again mistaken in the species. As previously stated, my figures of the first maturation division, metaphase, in *Acholla multispinosa,* agree in number and size relations with Montgomery's figures of the same stage in "*Sinea.*" Again his figures of "*Acholla multispinosa*" agree with my figures of the first division in *Acholla ampliata.* So it seems very probable that the species which he called "*Sinea diadema*" is *Acholla multispinosa,* and that his "*Acholla multispinosa*" is *Acholla ampliata.*

The behavior of the chromosomes in *Acholla multispinosa* is, in several respects, more remarkable than in any of the other species examined. The number of chromosomes in the female cells (Fig. 10, *A, B* and *C*) is 30, six of which are much smaller than the others. The male cells (Fig. 10, *D, E* and *F*) show 26 chromosomes, one of which is much larger and three much smaller than the remaining ones. At first sight, no evident relation is seen between the male and female groups. A relation becomes evident, however, from a study of the first and second maturation divisions. In the metaphase plate of the first division (Fig. 10, *H, I* and *J*) are 16 chromosomes. The three small ones are present and also the large one, which at this time is linked with two other chromosomes, one in contact with each end. This same grouping is present in the prophase of the first division (Fig. 10, *G*). Since there are 26 chromosomes in the

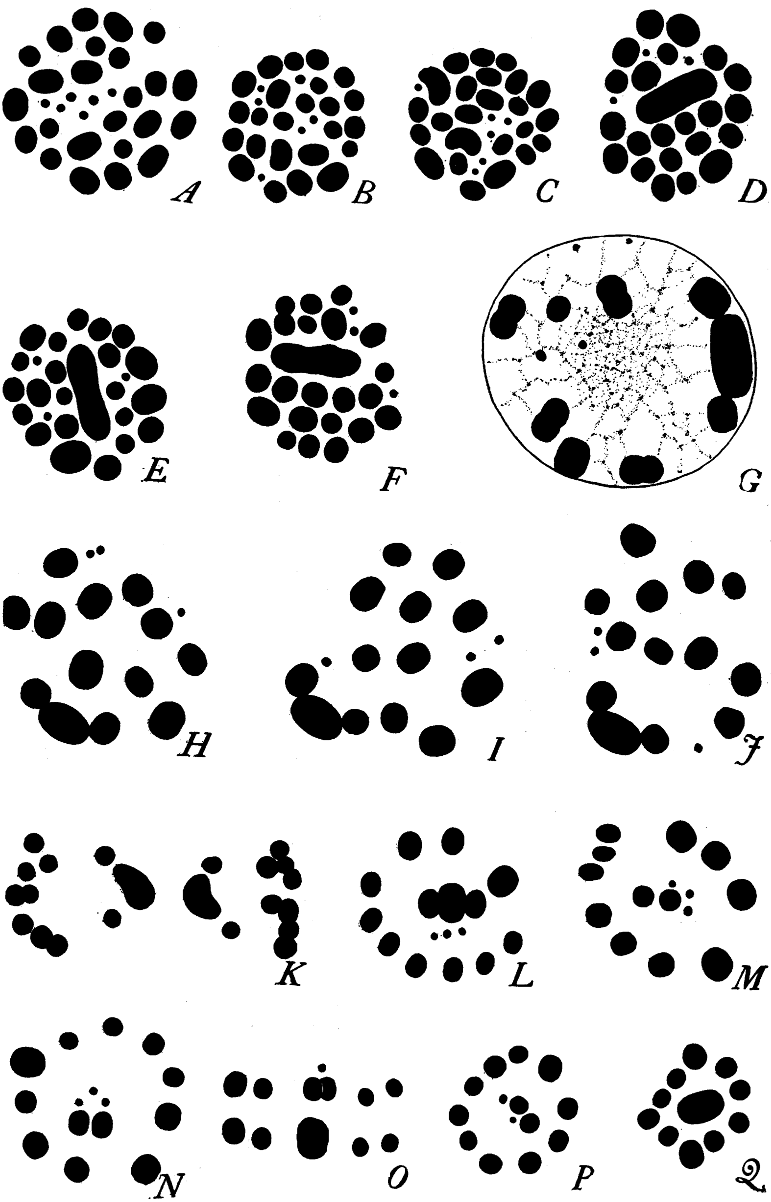


FIG. 10. *Acholla multispinosa* De Geer. *A–C,* female groups showing 30 chromosomes, six of which are small; *D–F,* male groups, 26 chromosomes, one of which is very large and three small; *G,* prophase of the first division showing the chromosome complex; *H–J,* metaphase figures of the first division with 16 chromosomes—the chromosome complex is still present; *K,* a side

spermatogonia and 16 in the first division, ten of these must be bivalent and six univalent. While I have not seen the anaphases showing the division of the three small chromosomes, it seems very probable that they and all the others divide equally in this division. The fact that 16 chromosomes are present in the equatorial plate of the second division, supports the above conclusion (Fig. 10, *L, M* and *N*; in *L* and *M*, the large chromosome which lies below the five in the middle, could not be shown). The chromosomes in the group of three formed by the large one and two others, lag behind and are the last to divide (Fig. 10, *K*, a side view of the first division, anaphase).

The metaphase plate of the second division again shows a regrouping of the chromosomes, similar in arrangement to those already described, but differing in the number of elements which compose the central group. Ten of the chromosomes form an irregular ring, within which are the other six, forming a hexad group (Fig. 10, *N*), composed of the three small chromosomes, the large one and the two which were linked with it in the first division. The three small and two medium sized ones lie approximately in one plane, while the large one lies a little above or below these five, on the other side of the equatorial plane. Unfortunately no side views of the metaphase showing all six members of the hexad group, were present in my material. The small chromosomes are so very minute at this stage that they are with difficulty seen at all. The chromosomes in the ring divide equally, while the members of the hexad group do not divide individually, but the group separates so that the large one passes to one pole and the remaining five to the other. Anaphases showing a clear demonstration of this separation were not present in my material. If there is any doubt as to the

view of the first division, anaphase, showing the late division of the complex; *L–N*, metaphase figures of the second division, showing the ring of ten bivalents and the univalents in the center—in *M* and *N* only five of the univalents are shown, as the large one lies beneath; *O*, side view of the second division, anaphase, showing the manner of separation of the large and two medium sized univalents—one small one is also present. No anaphases, showing the distribution of the three small univalents, were present. *P*, anaphase, polar view of the second division, showing the two medium univalents, and two of the small ones; *Q*, anaphase, showing the large univalent going to one pole. The enlargement is 4,018 diameters.

manner of the separation, it is in regard to the behavior of the small chromosomes. Fig. 10, *O*, a side view of an early anaphase, and Fig. 10, *P* and *Q*, pole views of the early anaphases, demonstrate clearly the behavior of the large and two medium sized ones. A number of anaphases showing one and two small chromosomes were present (Fig. 10, *O* and *P*), and when present, they were always with the two medium sized ones and not with the large one. A thorough study of many anaphases showing the large chromosome, has in no case, revealed the presence of a small one (Fig. 10, *P*). Further, the number of chromosomes and their size relations in the male and female cells makes it almost conclusive how the hexad group separates. As previously stated, the female group has 30 chromosomes, six of which are very small, and the male group has 26, three of which are small and one very large. If five members of the hexad group go to one pole and one to the other, two classes of spermatozoa are produced, containing 15 and 11 chromosomes respectively. The 15-chromosome class will contain 12 chromosomes of nearly equal size and three very small ones. The 11-chromosome class will contain ten chromosomes of approximately equal size and one very large one. Now, if the 15-chromosome class meets an egg with 15 chromosomes, three of which are small, the cells of the resulting individual will have 30 chromosomes, six of which will be small. This condition is fulfilled in the female chromosome group. If the same egg is met by the 11-chromosome class of spermatozoa, the cells of the offspring will contain 26 chromosomes, three of which will be small and one very large. This is the number and size relations found in the male cells. No other manner of separation of the hexad group could bring about a similar end result. It becomes evident then that the reduced number of chromosomes in the egg is 15, and that females are produced upon fertilization by the 15-chromosome class of spermatozoa; males upon fertilization by the 11-chromosome class.

Egg 15 plus spermatozoön 11 = 26 (♂),
Egg 15 plus spermatozoön 15 = 30 (♀).

As previously stated, the material which I have, does not

justify me in saying that the above statements are absolutely true, but all the data point in that direction. I hope to reëxamine the form as soon as material can be procured. If, then, these inferences prove true, *Acholla multispinosa* gives another new type of chromosome distribution in which the female has four more chromosomes than the male. For lack of material, I have not attempted to follow the differential chromosomes through the growth period.

It was noticed in the prophase and metaphase of the first division that three chromosomes (the large one and two others) were joined together end to end. Montgomery described such a complex in his account of the chromosomes in a species which he called "*Sinea diadema.*" In his first paper ('01) he considered the large chromosome representing two bivalents, but later ('06) described it as one large bivalent. All three members of this complex prove to be univalent in *Acholla,* since they divide only in the first mitosis.

In all previous cases where a dimorphism of the spermatozoa in regard to the number of chromosomes exists, the female has not only received the larger number of chromosomes, but as far as we can judge with the eye, the larger amount of chromatin. While the numerical rule still holds, *Acholla* seems to give an exception in that the quantity of chromatin which goes to the male-producing pole is greater than that which goes to the female-producing one. I have made a number of measurements in the first and second divisions, and in every case, the large chromosome seems to contain a larger amount of chromatin than the remaining five differential chromosomes. In connection with the question of sex-determination, this point will be discussed further.

What can be the significance of such a chromosome complex as is present in the first maturation division and in the prophase of this division? In the Phasmidæ, Sinety ('01) recorded a coupling of the odd chromosome with one of the ordinary bivalents. McClung ('05) extended this observation to two other families of Orthoptera, the Acrididæ and Locustidæ. Wilson ('07) describes a coupling of the supernumerary chromosome with one of the idiochromosomes in *Metapodius* and suggests the possibility that such chromosome couplings may give

the physical basis of certain forms of correlation in heredity. The suggestion seems a very plausible one for the above cases, but the complex in *Acholla* is somewhat different. All three chromosomes are univalent and divide in the first division. In the second division the group separates, the large chromosome passing to one pole and the other two to the opposite pole.

Acholla ampliata Stal.

Acholla ampliata appears to be the species which Montgomery ('01 and '06) describes under the name of "*Acholla multispinosa.*" He figures the spermatogonial division with 32 chromosomes, eight of which are very small, and both the first and second divisions with 16 chromosomes, four of which are small. In the first division, the four small ones may lie in any position, but in the second division, they are centrally placed and grouped closely together. Unfortunately my material shows only the first and second divisions which agree with those, which Montgomery figures, except that in the second division, the small ones are grouped so closely together that it is impossible to distinguish

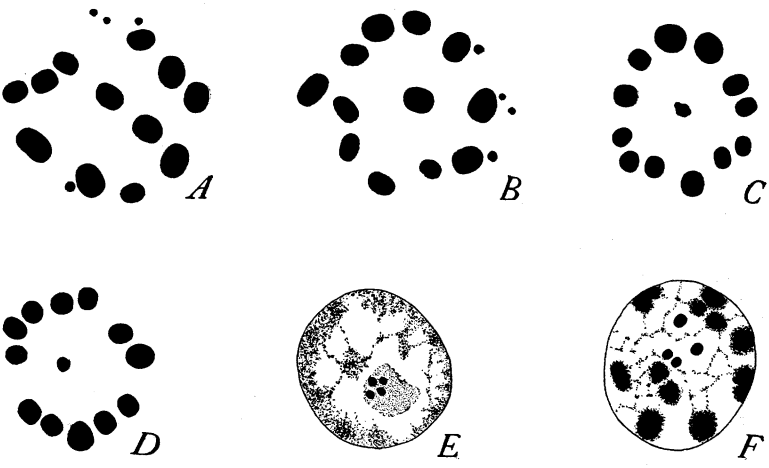
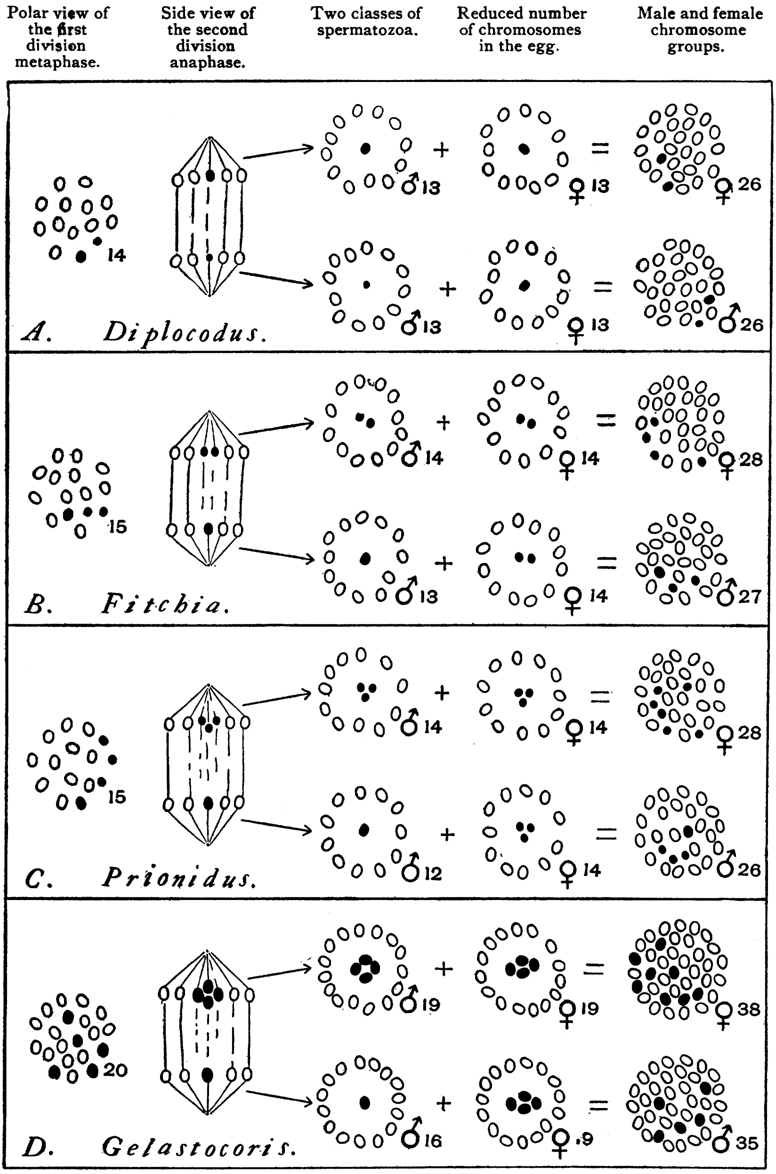


FIG. 11. *Acholla ampliata* Stal. *A* and *B*, metaphase figures of the first division, 16 chromosomes; *C* and *D*, metaphase figures of the second division —there are 12 chromosomes in the ring and the small ones are grouped in the center so that it is impossible to count them; *E*, typical appearance of the nucleus during the later part of the growth period—the four small chromosomes are embedded in the plasmosome; *F*, prophase of the first division—the plasmosome has disappeared, but the four small chromosomes remain. The enlargement is 4,018 diameters.


Polar view of the first division metaphase.
Side view of the second division anaphase.
Two classes of spermatozoa.
Reduced number of chromosomes in the egg.
Male and female chromosome groups.
14
♂13 + ♀13 = ♀26
♂13 + ♀13 = ♂26
A. Diplocodus.
15
♂14 + ♀14 = ♀28
♂13 + ♀14 = ♂27
B. Fitchia.
15
♂14 + ♀14 = ♀28
♂12 + ♀14 = ♂26
C. Prionidus.
20
♂19 + ♀19 = ♀38
♂16 + ♀19 = ♂35
D. Gelastocoris.

the number or arrangement with certainty. This difference, however, could be easily accounted for by a difference in fixation, as I have noticed the same thing in regard to the tetrad group in *Sinea*. As he found 32 chromosomes in the spermatogonia, Montgomery thought that the four small ones divided equally in both divisions, and he figured them as dyads attached to the plasmosome during the growth period. I have no evidence to offer to the contrary, but one thing comes out clearly in a study of the growth period, and that is that the four small chromatin bodies which Montgomery calls "chromatin nucleoli," show no appearance of being bivalent, and that they are not merely in contact with the plasmosome, but are actually embedded in it (Fig. 11, *E*). In the prophase of the first division (Fig. 11, *F*), the plasmosome breaks down and the small chromosomes remain, but even here they do not appear to be bivalent.

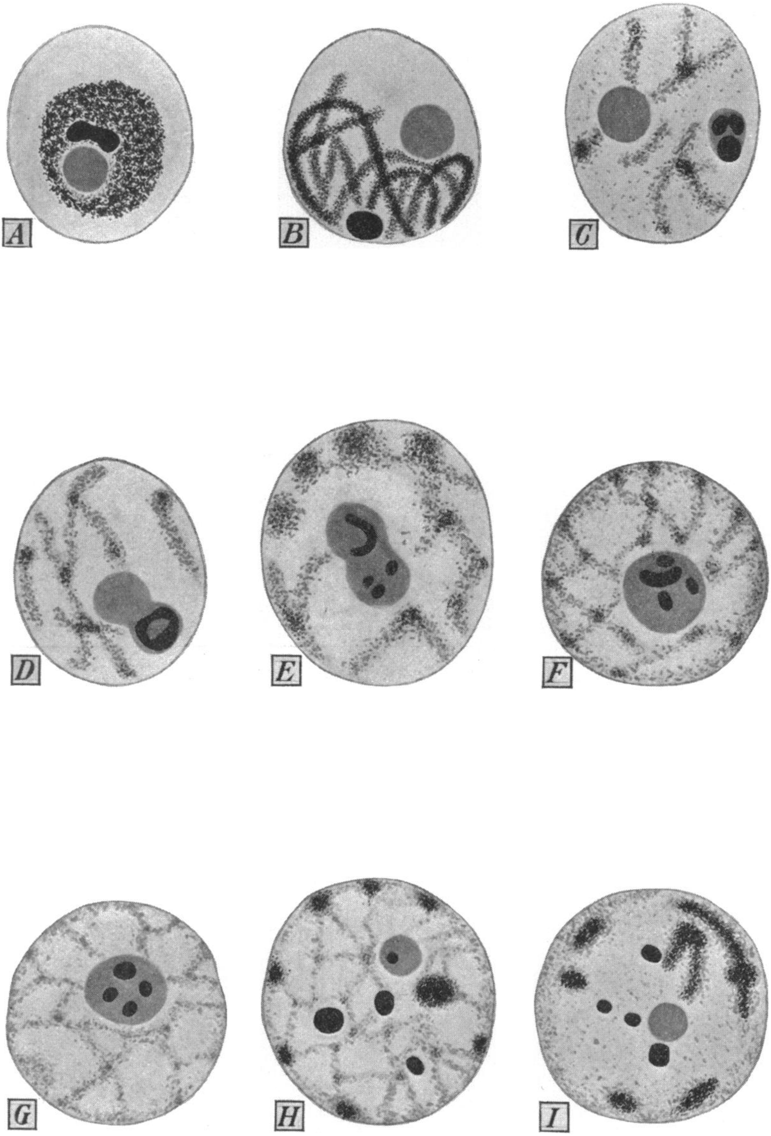
In comparing Fig. 11, *E* and *F*, with Fig. 6, *M* and *N*, the same stages in *Sinea diadema*, we notice a striking similarity. As the four small chromosomes embedded in the plasmosome in *Sinea* are univalent, is it not also possible that the same is true in *Acholla?* In the light of the present discoveries, it seems desirable that the form be reëxamined.

In order to summarize briefly the results, I have made a diagram (Fig. 12) illustrating the four different types of chromosome distribution described in the present paper. I have omitted from the diagram the type described in *Acholla multispinosa* as my evidence is hardly conclusive. The differential chromosomes are in each case made in black, while the remaining ones are left in outline. The size relations of the differential chromosomes are maintained as nearly as possible. In the first column to the left, are the metaphase figures, polar view, of the first division in each type. In the second column are side views of the second division, anaphase, showing the manner of separation of the dyad, triad, tetrad and pentad groups of differential chromosomes. The third column gives the two classes of spermatozoa produced in each type; the fourth, the reduced number of chromosomes in the egg (inferred); and the fifth, the end result produced by the fertilization of the egg by the two classes of spermatozoa.

(To be continued.)

Explanation of Plate I.

Prionidus cristatus Linn. These drawings illustrate the history of the differential chromosomes and the plasmosome during the growth period. *A,* about the contraction-phase of synapsis—the plasmosome and a deeply staining chromatic body is present; *B,* the spireme stage following synapsis; *C,* the chromatic body is breaking up and a plasmosome is forming around it; *D,* the stage following *C*—the two plasmosomes have commenced to fuse; *E,* the chromatic element breaks up for the first time into its constituent elements, the differential chromosomes; *F* shows the complete fusion of the two plasmosomes; *G* gives the typical appearance of the plasmosome and differential chromosomes during the greater part of the growth period—the larger differential chromosome has contracted; *H,* a prophase of the first division—the plasmosome has grown smaller and the differential chromosomes are coming out; *I,* prophase of the first division, where all the differential chromosomes have left the plasmosome. *A, B, C, D* and *E* are enlarged 4,018 diameters; *F, G, H* and *I,* 2,594 diameters.


A
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